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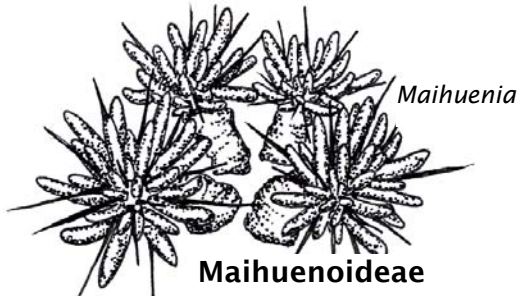
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Cladistics of the Cacti

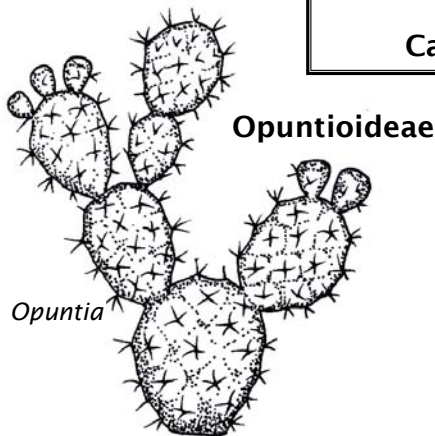
by

Ruth Kirkpatrick, Abby Moore, Bianca Knoll, Vicente Garcia, Anna Larsen,
Andy Murdock, and Michael Park

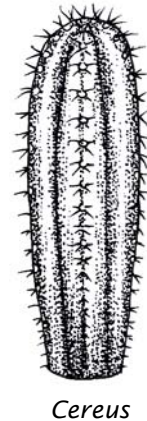
(revised 2008 by Eric Harris; 2009 by Steph Stuart and Nick Matzke)



Traditional Cactaceae Subfamilies



Cactoideae



Things to do Before Lab

1. Read "Introduction to Cladistic Analysis" and pages 1-3 of this handout **before** coming to lab.
2. Make sure that you're familiar with the terms and concepts listed below before coming to lab. Most of them are defined in the "Introduction to Cladistic Analysis."

Linnaean classification

Phylogenetic

classification

Cladogram

Phylogeny

Clade

Polytomy

Taxon (plural taxa)

Monophyletic

Polyphyletic

Paraphyletic

Outgroup

Ingroup

Derived

Ancestral

Character state

Apomorphy

Synapomorphy

Autapomorphy

Plesiomorphy

Symplesiomorphy

Homoplasy

Reversal

Introduction

Phylogenetic and Cladistic Methods

Systematics is the classification of living organisms into a hierarchical series of groups emphasizing their evolutionary relationships (a.k.a. **phylogeny**). **Cladistic methods** use recency of common ancestry to group taxa and generate phylogenetic hypotheses. These relationships are then used as a basis for classification, so that all recognized taxa constitute **clades**, or **monophyletic** groups.

The only problem is that, compared to classification, cladistic methods are relatively new. Classification has existed for many years, since Linnaeus' first work in 1735. Cladistics was invented much later, by Hennig in 1950. Hennig's major contribution was to realize that similarities between taxa fall into three different groups: synapomorphies, symplesiomorphies, and homoplasies. By figuring out which characters are synapomorphies and using only those to group taxa, we can tell which groups are clades. Until cladistics was invented, there was no scientific way of telling what the evolutionary history of any group was. Instead, **Linnaean classification** used all kinds of similarity to group taxa. This included homoplasies and symplesiomorphies along with synapomorphies. Because of this, some groups that were made in the past have turned out not to be monophyletic. Today, an effort is underway to make sure all named groups are monophyletic.

One of your tasks today will be to look at the traditional classification of cacti. We now know that the cacti are monophyletic. Many groups within the cacti, however, may have been classified into paraphyletic or polyphyletic groups. As you work, ask yourself: which characters were wrongly identified as synapomorphies that grouped these plants together? How does the phylogeny change once we separate shared, derived characters from all shared characters? What can we learn about the way evolutionary change happens once we understand how the characteristics typical of cacti have changed through time?

About the Cacti

The Cactus Family (Cactaceae) includes approximately 1500-1800 species distributed throughout the North and South America. Cacti show remarkable morphological and physiological adaptations to drought, and represent one of the world's most spectacular desert radiations.

Most cacti are adapted to living in dry environments in at least four ways:

- (1) by photosynthesizing with their stems, instead of leaves
- (2) by having spines that protect their stored water and photosynthate
- (3) by storing water, when it is available, in deeper tissues of their stems
- (4) by using a water-conserving form of photosynthesis

Where did this amazing group come from, though? How did they make the transition from being normal plants, with leaves, bark, and no thorns, to the spiny, succulent plants we know today? During this lab, we will use phylogenetic techniques to explore the evolutionary history of these transitions.

It turns out that there are some cacti still around that do have leaves. Two genera are especially notable: *Maihuenia* (pronounced “Mao-hen-ee-ah”) and *Pereskia*. *Maihuenia* consists of two species that live in cold, dry regions of Patagonia and Chile. They grow in dense stands of short stems with leaves crowded at the tips. *Pereskia* species are widely distributed in the Caribbean and Central and South America in a range of warm and seasonally dry forest habitats. Unlike most cacti, *Pereskia* live in moister environments and have broad and flat leaves, and non-photosynthetic, non-succulent stems. Sometimes, *Pereskia* and *Maihuenia* have been grouped together based on their shared characteristics. Today, we’ll be examining whether these similarities are really a good indicator of shared evolutionary history.

Does either group represent the ancestral state of cacti? In this lab, we will be exploring this question. We’ll be comparing these two cactus taxa to other, more familiar cacti, including Prickly Pear (*Opuntia*) and Barrel Cactus (*Echinocactus*). These “typical” cacti are recognized by their stem succulence, ephemeral leaves or complete absence of green leaves and well-developed photosynthetic stems. We’ll also be looking at an outgroup of the cactus clade, *Anacampseros telephiastrum*.

Although each of the cacti we’ll be looking at today has distinct characteristics, they share the synapomorphy of **spines**. Therefore, **PLEASE BE CAUTIOUS OF SPINES WHEN HANDLING THE CACTI!**

Guide to Morphological Characters of the Cacti

Stem Morphology and Areoles

All the members of the Cactaceae produce two distinct types of stems called “**long shoots**” and “**short shoots**”. A long shoot is simply a stem with long **internodes**. Short shoots are stems with much shorter internodes. In cacti, the short shoots have become so short that they are reduced to just a dot on the long shoot. The short shoots of cacti have especially short internodes and are called **areoles**.

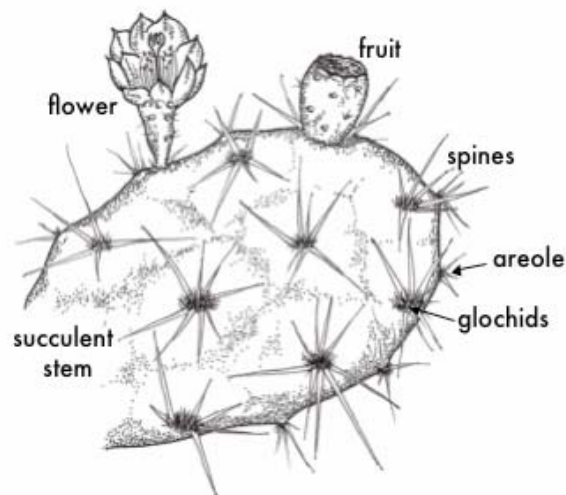


Figure 1. *Opuntia* (prickly pear cactus) showing typical morphological characteristics of the Opuntioideae.

Guide to Morphological Characters of the Cacti – continued

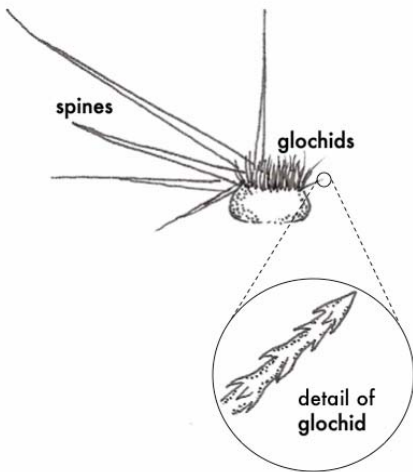


Figure 2. Areole with spines and tuft of glochids.

Spines and Glochids

Spines (hard, spiky modified leaves) and in some taxa and **glochids** (barbed hairs) grow from the areoles. See the labeled **plant body** and cactus body diagrams below (Figures 1 and 2). Be especially careful of the glochids, as they break off in your skin and can be very painful and hard to remove.

Flowers

Flowers also grow from the areoles. Cactaceae flowers are distinctive. They are perfect and possess many showy and spirally arranged sepals and petals that look alike. Their flowers also have ovaries that are deeply recessed into the apex of modified stems, so the outer portion of a cactus ovary is covered with spine-bearing areoles. See the labeled diagram of a typical cactus flower (Figure 3).

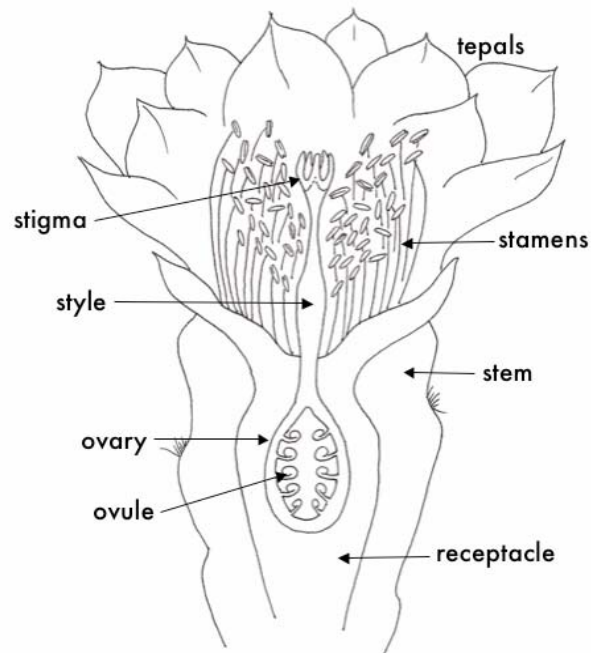


Figure 3. Cross-section of *Opuntia* (prickly pear) flower showing the ovary deeply recessed into the stem.

Your Goals

In this lab, your team will conduct a cladistic analysis of cactus species that represent a cross-section of the morphological diversity found in the Cacti, and then use this phylogeny to examine how changes have come about in this group.

The steps in today's lab are:

- 1) Use morphological characters to search for synapomorphies, and use them to build clades.
- 2) Add additional information from gene sequences to further resolve your phylogenetic hypothesis.
- 3) Use the cladogram to test different hypotheses about relationships within the cacti, and answer questions about the order of character evolution in this group.

****Instructions for the lab write-up are given at the end of the handout****

OUTGROUP = close relatives of Cactaceae

Portulacaceae (Purslane Family)

Anacampseros telephiastrum (“Pan American Love Plant”)

INGROUP = Cactaceae (Cactus Family)

TRADITIONAL SUBFAMILIES

Cactoideae <i>Cereus</i> sp. (“Cereus”) <i>Echinocactus</i> sp. (“Barrel Cactus”)	Opuntioideae <i>Austrocylindropuntia subulata</i> (“Cane Cholla”) <i>Opuntia longispina</i> (“Prickly Pear”) <i>Quiabentia verticillata</i> <i>Tephrocactus glomeratus</i> (“Paper Spine Cactus”) <i>Peresklopsis porteri</i> (“Alcajer”)
Maihuenioideae <i>Maihuenia poeppigii</i> (“Chupa Sangre”)	Pereskioideae <i>Pereskia aculeata</i> (“Barbados Gooseberry”) <i>Pereskia bleo</i> (“Wax Rose”) <i>Pereskia grandifolia</i> (“Rose Cactus”)

PERFORMING A CLADISTIC ANALYSIS OF THE CACTUS FAMILY

Obtain for your lab group one set of potted cactus (**ingroup**) and Portulacaceae (**outgroup**) species and one binder with supplemental photographs of these species.

I. Cladistic analysis using morphological data

Use the charts and boxes on the following pages to carry out a cladistic analysis of the cactus species. Labeled diagrams, observations of living plants, and your GSI will help you interpret the morphology and determine the character states for each cactus and outgroup species.

Step 1: Group the pots of ingroup taxa & outgroup taxa to keep things clear.

Step 2: Examine the morphology and select character states of the ingroup & outgroup taxa.

Step 3: Determine whether each character state is ancestral (a plesiomorphy) or derived (an apomorphy) (“0” indicates ancestral and “1” or “2” represent various derived character states). This determination has been completed for you. Work with your group members to complete the following matrix of morphological characters of your ingroup and outgroup taxa.

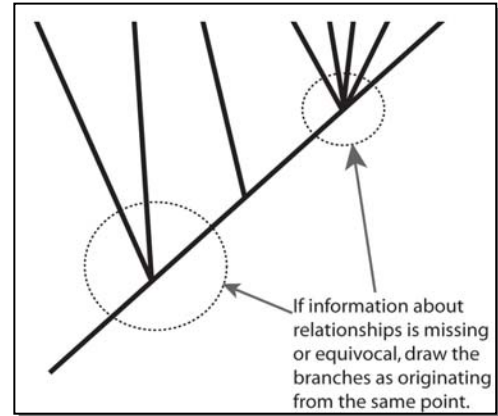
Taxa:		1	2	3	4	5	6	7	8	9	10	11	12
Characters:		Succulent Tissues, 0 = present, 1 = absent	Spines, 0 = absent, 1 = present	Ovary Position 0 = normal, 1 = recessed into stem	Areoles, 0 = absent, 1 = present	Leaves, 0 = present, 1 = reduced &/or ephemeral, 2 = absent	Glochids, 0 = absent, 1 = present	Ribbed Stems, 0 = absent, 1 = present	Stem stomata, 0 = absent, 1 = present	Delayed bark, 0 = absent, 1 = present	Majority of photosynthesis performed by, 0 = leaves, 1 = stem	Areoles give rise to leaves, 0 = yes, 1 = no	Habitat, 0 = mesic & seasonally dry, 1 = hot & dry, 2 = cold & dry
A	<i>Anacampseros telephiastrum</i>			0			0		0	0		-	0
B	<i>Pereskia bleo</i>			1			0		0	0		0	0
C	<i>Pereskia aculeata</i>			1			0		1	0		0	0
D	<i>Pereskia grandifolia</i>			1			0		1	1		0	0
E	<i>Opuntia longispina</i>			1		1	1		1	1		0	1
F	<i>Quiabentia verticillata</i>			1			1		1	1		0	1
G	<i>Pereskiopsis porteri</i>			1			1		1	1		0	1
H	<i>Tephrocactus articulatus</i>			1		1	1		1	1		0	1
I	<i>Austrocylindropuntia subulata</i>			1		1	1		1	1		0	1
J	<i>Maihuenia poeppigii</i>			1			0		0	0		0	2
K	<i>Echinocactus</i> sp.			1			0		1	1		1	1
L	<i>Cereus</i> sp.			1			0		1	1		1	1

Step 6: Circle shared derived characters in each column to recognize and form clades.

Step 7: Convert data matrix into Venn Diagrams (nested boxes) of subclades and clades based on shared derived characters, starting with the most inclusive groups.

Step 8: Draw and connect branches below the Venn diagram and transform taxon relationships into a branching diagram (cladogram) with the taxa at the tips.

NOTE: If there is no difference between taxa based on your data, you can use a **polytomy** to show uncertainty and group them together at a single node. Draw those taxa branching from the same point, as indicated in the figure to the right.



Venn Diagram

Cladogram

Questions for morphological analysis

- 1) In this analysis, which characters were synapomorphies of the cacti (that is, shared by all taxa except for *Anacampseros*?)
- 2) Which characters do *Pereskia bleo* and *Maihuenia* share that grouped them together? Indicate whether the character was present or absent.
- 3) Did *Pereskia bleo* and *Maihuenia* share more presences, or absences?
- 4) Do any of the *Pereskia* taxa have the exact same set of synapomorphies?
- 5) Based on your morphological data, there are two places where the evolutionary relationships between the taxa are not clear. You should have indicated this with a polytomy. List the taxa in each polytomy. (You may list them by letter to make your answer shorter.)

II. Building a cladogram based on combined data

How do we find the phylogeny that shows the evolutionary relationships between the taxa in the ingroup? Cladistic researchers assume that evolution happens slowly, one step at a time. Therefore, a cladogram that proposes that a change happened just once is considered more likely than one that assumes that the change occurred separately many different times. This is known as the principle of parsimony. According to this principal, the simplest explanation is the one that is most likely to be right. It is hard to compare all possible cladograms by hand for a large data set. As a result, most cladists use computer programs for this task.

Although morphological characters are often used in modern cladistic analysis, they are usually combined with molecular characters from DNA, protein, or genome sequences. Because gene sequences are long, they offer many more characters. Genes also experience selection differently from the phenotypic characters we looked at in the first part of the lab. Therefore, by combining both types of characters, we have a better chance of getting around any convergent evolution that would lead us to mistake homoplasy for synapomorphy.

Gene data can take a long time to collect, extract, and analyze. Luckily, Edwards et al. (2004) already collected sequence data for this group. We'll use these data, along with the morphological data you collected, to refine our phylogenetic hypothesis.

Your GSI will input data that includes both the morphological characters you collected and DNA data derived from the work of Edwards et al. into Phylip pars (pars stands for 'parsimony'), a program written by Felsenstein (1989) that is available for free use online from Mobyly (Néron et al. 2008). The program will analyze all the data together, and use the principle of parsimony to find the tree that minimizes number of changes on the cladogram. Similarities that fit with this pattern are considered synapomorphies. Similarities that do not fit with this pattern will be discarded, because they are considered symplesiomorphies or homoplasies.

Once the program is done running, we will use the Dendroscope program (Huson et al. 2007) to view the phylogeny it has found. Your GSI will then hand out copies of this cladogram that you can use in the next part of the lab.

Questions for building a cladogram

- 6) Fill out this chart with "yes" or "no" to indicate which types of similarity are used to build a cladogram and which were used in traditional classification.

Type of similarity	Used to build cladograms?	Used in traditional classification?
Apomorphy		
Synapomorphy		
Symplesiomorphies		
Homoplasy		

- 7) What is the principal of parsimony?
- 8) How do phylogenetic computer programs decide which similarities are synapomorphies?

III. Mapping character data onto the cladogram

Step 1: Obtain a cladogram Your GSI will give you a copy of the final cladogram generated by using combined molecular and morphological data.

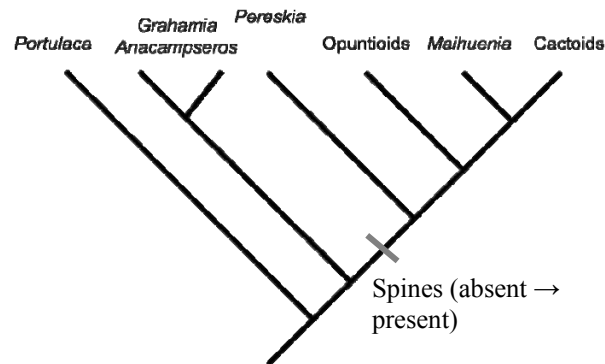
Step 2: Choosing characters of interest How did the character states that make cacti unique evolve? Think back to the list of characters from the introduction to this lab. It included three unique features that helped cacti adapt to dry environments:

- (1) Photosynthesizing with their stems, instead of leaves
- (2) Spines that protect their stored water and photosynthate
- (3) Storing water, when it is available, in deeper tissues of their stems

These correspond to the characters:

- (1) Stem stomata, 0 = absent, 1 = present
Delayed bark, 0 = absent, 1 = present
Majority of photosynthesis performed by, 0 = leaves, 1 = stem
Leaves, 0 = present, 1 = reduced or ephemeral, 2 = absent
- (2) Spines, 0 = absent, 1 = present
- (3) Succulent Tissues, 0 = present, 1 = absent

The character of succulent tissue is present in the outgroup, so we won't be mapping it on the cladogram. However, we will map the other five characters, since they correspond to important adaptations in cactus evolution.

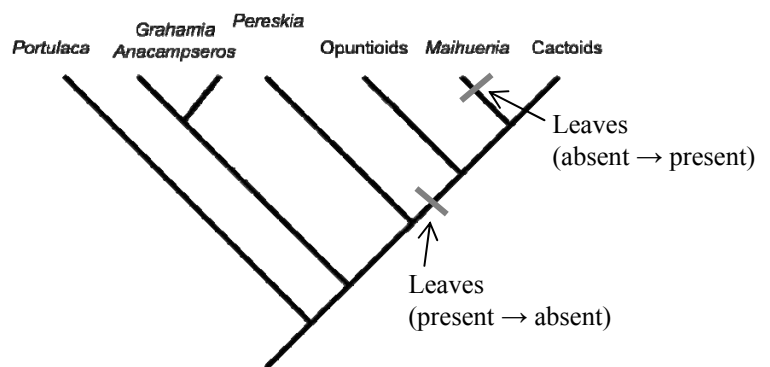


cladogram could be different.) This shows that spines are a synapomorphy of all the taxa above this line, and that the absence of spines is a symplesiomorphy of the taxa below this line.

But what if a character changed twice? In this case, you will need to map both changes on. For instance, if leaves were lost after *Pereskia*, but regained by *Maihuenia*, you would map this as shown

Step 3: Mapping how characters changed

Now, you can look at the evolutionary order of character changes in the cacti, using the data you collected in Part I, and the class tree from Part II. Let's start with the character of spines. Spines changed only once, from absent to present, between *Anacamperos* and *Pereskia*. So, to map the character spines on to the tree, we would make a mark on the cladogram between these two taxa, and label it as shown. (This cladogram is just an example. Your final



here. You should minimize the number of changes, in accordance with the principal of parsimony.

Step 4: Looking at all the changes together How did the characters that make cacti unique evolve? Did these characters evolve all at once, or one by one?

Questions for character mapping

- 9) Choose one clade from your cladogram and list the morphological synapomorphies that unite it. You can use characters that you did not map as well as ones you did.

- 10) Based on your observations, can you infer where, in the evolution of cacti, the following traits evolved:
 - i) Spines

 - ii) Stem photosynthesis

 - iii) Loss or gain of leaves

- 11) Spines, stem photosynthesis and lack of leaves are often thought of as the defining characters of cacti. But do you see any evidence of transitional forms – in other words, cacti that have some, but not all, these characters? Which taxa are transitional?

Map Questions: Distribution of Cactus Taxa

- 12) Finish labeling the cladogram on the “Distribution of Cactus Taxa” map. Fill in each of the empty boxes with the name of the cactus taxon that belongs there, according to your phylogenetic results.
- 13) Using the completed “Distribution of Cactus Taxa” map and the “Rainfall Map of South America,” explain what environment each cactus taxon is found in. Use the location of the dark dot at the end of each cladogram branch to estimate the climate where that taxon is found. Assume that temperature decreases as you move away from the equator. You may want to mark the location of each taxon on the rainfall map.

Taxon	Rainfall	Temperature
<i>Anacampseros telephiastrum</i>	<i>Medium</i>	<i>Moderate</i>
<i>Pereskia bleo</i>		
<i>Pereskia aculeata</i>		
<i>Pereskia grandifolia</i>		
<i>Opuntia longispina</i>	<i>Dry</i>	<i>Hot</i>
<i>Quiabentia verticillata</i>	<i>Dry</i>	<i>Hot</i>
<i>Pereskiopsis porteri</i>	<i>Dry</i>	<i>Hot</i>
<i>Tephrocactus articulatus</i>		
<i>Austrocylindropuntia subulata</i>		
<i>Maihuea poeppigii</i>		
<i>Echinocactus</i> sp.	<i>Dry</i>	<i>Hot</i>
<i>Cereus</i> sp.		

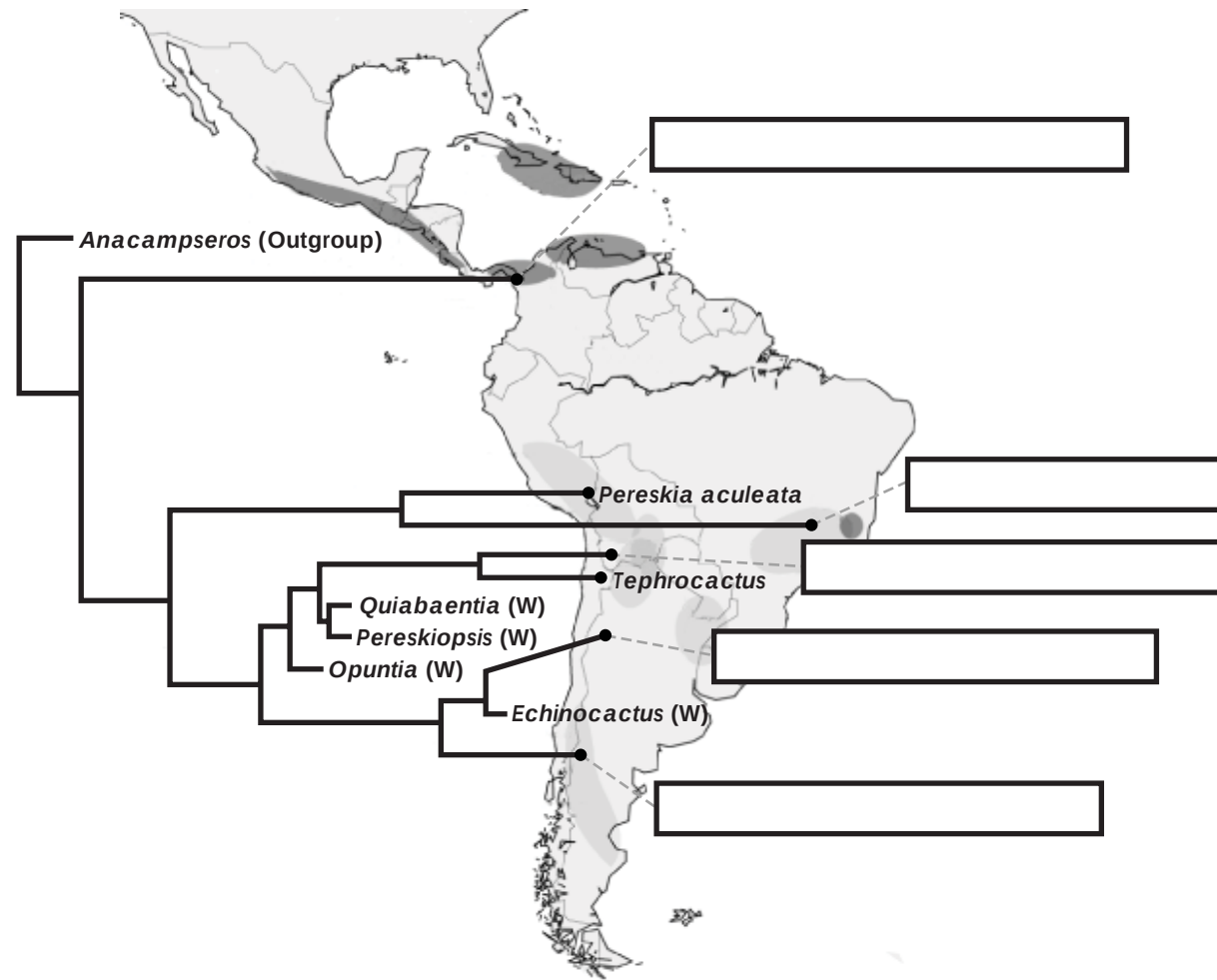
- 14) What trends do you see in cactus evolution? Which of the characters you mapped on the tree developed in a warm, wet environment? Which developed in a hot, dry environment?

Distribution of Cactus Taxa

This map combines the cactus phylogeny with information showing where cactus specimens for each taxon were collected. (W) indicates that the taxon is widespread and occurs in many different arid regions.

Shaded areas indicate the range of different cactus taxa.

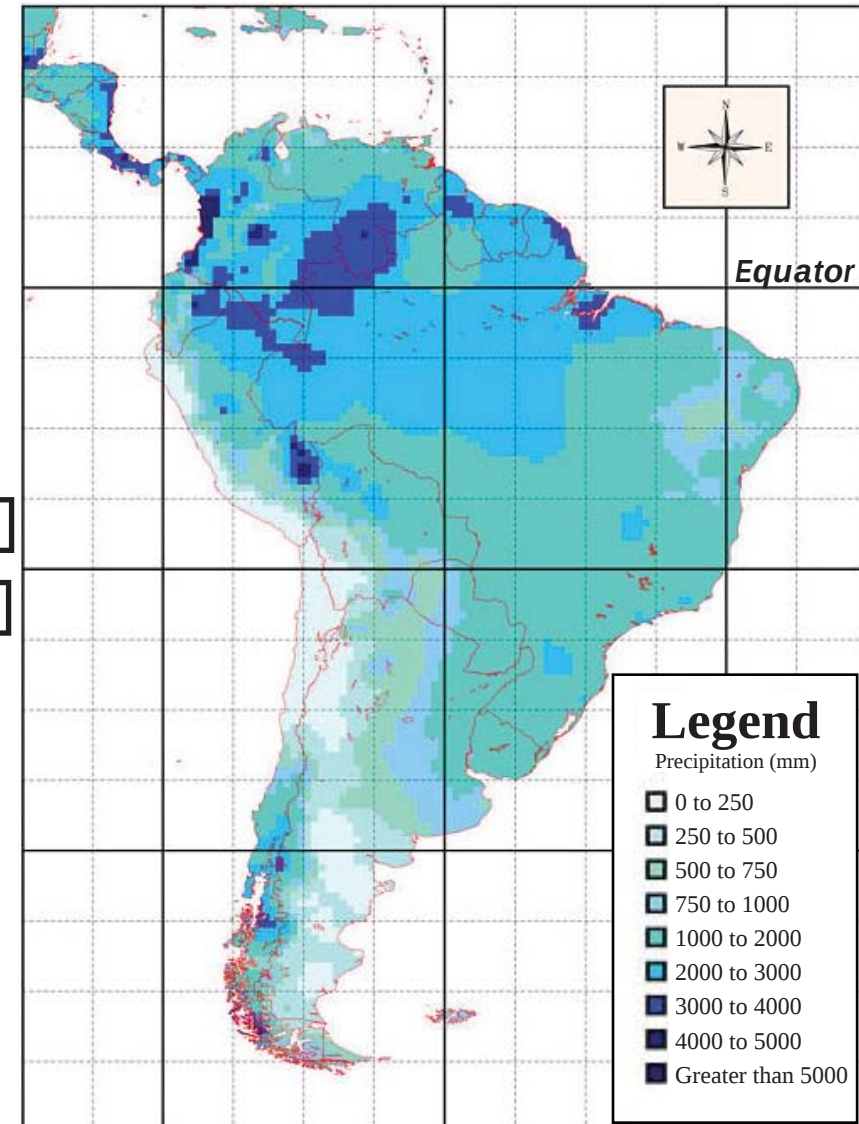
Use the phylogeny you developed to finish filling in this chart.



Rainfall Map of South America

Darker colors indicate higher rainfall.

In general, temperatures are higher near the equator.



Concluding Questions

- 15) Based on the evolutionary hypothesis represented by the cactus phylogeny, what do you think the common ancestor of all the Cacti looked like? What environment did it grow in?
- 16) Is *Pereskia* a monophyletic group? Where there any hints in the morphological data that suggested this conclusion?
- 17) Do you think *Maihuenia* should be grouped with *Pereskia*? Explain why the similarities between the two groups do, or do not, represent synapomorphies.
- 18) *Maihuenia* lives in cold, dry regions in Patagonia and Chile. They grow in dense stands of short stems, which serve as a thermal buffer against freezing temperatures. How might the dense crowding have selected for persistent leaves and non-photosynthetic stems?

Lab Write-Up

Using the cladogram that you constructed from morphological data as well as the cladogram that we obtained from the combined data, complete one of the write-up options below. Include all cladograms when you hand your report.

Option A

Answer the questions in this handout, and turn them in, along with:

- 1) Your morphological data matrix
- 2) Your morphological cladogram and Venn Diagram
- 3) The final cladogram you mapped the characters on
- 4) The labeled “Distribution of Cactus Taxa” Map

Option B

Answer the following questions (answers should be in the form of a short paragraph, ~100-150 words long):

1. a) Are each of the four subfamilies monophyletic? If not, which one(s) are not? Based on the results of your cladistic analysis of the Cactus family, would you accept or reject the traditional hypothesis of relationship (e.g. the four subfamilies)?
 b) Is the genus *Pereskia* monophyletic, paraphyletic, or polyphyletic? Do your results support the “*Pereskia* model” of cactus evolution?
2. Why do you think there is a difference in the cladograms produced from morphological data alone and from morphological and molecular data together? Do you think one type of data is more reliable than the other? Why or why not?
3. Which taxon in the morphology-based tree is in a totally different location as compared to the combined analysis tree? Can you suggest and describe any adaptive hypotheses based on the environmental conditions of this taxon’s habitat?
4. Identify and explain an evolutionary trend of one of the morphological characters examined during the evolution of the Cactus family.
5. If you could have any additional character data to further refine your hypotheses of cactus evolution, what type of character data would you collect? Justify your answer.

Option C

Using a format specified by your GSI, write-up the results of your lab in the style of a scientific report.

Literature used in the development of this lab exercise:

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- Ruth Kirkpatrick: project leader, lab exercise and glossary composition, final editor and lab exercise design.
- Abby Moore: data matrix composition, lab exercise and glossary editing and composition.
- Bianca Knoll: instructional mini-grant composition, lab exercise and glossary editing and composition, lab exercise and glossary illustrations.
- Andy Murdock: acquisition of live plants and lab exercise editing.
- Vicente Garcia: Cactus Biogeography supplemental exercise and Cactus Photo Library.
- Michael Park: initial Venn diagram design and key.
- Anna Larsen: instructional mini-grant composition and lab exercise editing.
- Dr. Dean Kelch: acquisition of live plants and culture consultant.
- Drs. Glenys Thomson, Montgomery Slatkin, John Latto, and Brent Mishler: faculty sponsors and project reviewers.
- Eric Harris: revision of the lab exercise, 2008.
- Stephanie Stuart: addition of molecular data exercise, 2009.
- Nick Matzke: addition of molecular data exercise, 2009.